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Request for grant of a Patent 9820956.2

Form 1/77 **Patents Act 1977**

❶ Title of invention

1 Please give the title of the invention: Vaccine

25 SEP 1998

❷ Applicant's details

❑ **First or only applicant**

2a If you are applying as a corporate body please give:

Corporate Name: SmithKline Beecham Biologicals s.a.

Country *(and State of incorporation, if appropriate)*: Belgium

❷ Do not give trading styles, for example, 'Trading as XYZ company', nationality or former names, for example, 'formerly (known as) ABC Ltd' as these are not required.

2b If you are applying as an individual or one of a partnership please give in full:

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2c **In all cases,** please given the following details:

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3a Have you appointed an agent to deal with your application?

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❶

please give details below

Agent's name M J W DALTON

Agent's address SmithKline Beecham
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Agent's ADP number 412231300 6

3b: *If you have appointed an agent, all correspondence concerning your application will be sent to the agent's United Kingdom address.*

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❹ Reference number

4. Agent's or applicant's reference number *(if applicable)* RE/RH/B45156

Claiming an earlier application date

5. Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

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❻ Declaration of priority

If you are declaring priority from a PCT Application please enter 'PCT' as the country and enter the country code (for example, GB) as part of the application number

6. If you are declaring priority from previous application(s), please give:

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❼ Inventorship.

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•there is an inventor who is not
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Yes ☐ No ☒ ➲ ***A Statement of Inventorship on Patents form 7/77 will need to be filed (see Rule 15).).***

❽ Checklist

❽ Please supply duplicates of claim(s), abstract, description and drawings).

8a Please fill in the number of sheets for each of the following types of document contained in this application

Continuation sheets for this Patents Form 1/77 [–]

Claim(s) 3 Description 22

Abstract [ ] Drawing(s) 10

8b Which of the following documents also accompanies the application?

Priority documents *(please state how many)* [–]

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Patents Form 9/77 – Preliminary Examination Report [–]

Patents Form 10/77 – Request for Substantive Examination [–]

❾ Request

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I/We request the grant of a patent on the basis of this application.

Signed Marcus J W Dalton Date: 25/09/98

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Vaccines

The present invention relates to an adjuvant composition comprising a polyoxyethylene ether or a polyoxyethylene ester, in combination with a pharmaceutically acceptable excipient, and to a vaccine comprising such adjuvant compositions and antigen. In addition, the present invention relates to the use of polyoxyethylene ethers or esters in the manufacture of an adjuvant formulation, and to their use in the manufacture of vaccine formulations.

Mucosal vaccination, for example intranasal and oral, may represent an easy and more convenient way of vaccination than traditional vaccination through systemic injection. The use of an injection to administer a vaccine dose is associated with a number of disadvantages, namely pain and irritation at the injection site following injection. These factors may lead to "needle-fear" which has been known to result in poor patient compliance for vaccination regimes. Furthermore, conventional systemic injections can be a source of infection in the region of the skin puncture.

Apart from bypassing the requirement for injection, mucosal vaccination is attractive since it has been shown in animals that mucosal administration of antigens has a greater efficiency of inducing protective responses at mucosal surfaces, which is the route of entry of many pathogens. In addition, it has been suggested that mucosal vaccination, such as intranasal vaccination, may induce mucosal immunity not only in the nasal mucosa, but also in distant mucosal sites such as the genital mucosa (Mestecky, 1987, Journal of Clinical Immunology, 7, 265-276; McGhee and Kiyono, Infectious Agents and Disease, 1993, 2, 55-73).

In order for mucosal immunisation to be a viable replacement for, or alternative to, immunisation through injection, this vaccination route will have to be able to induce systemic immunological responses at least as efficiently as those induced by injection. While it has been reported that certain antigens when administered via this route are able to induce systemic responses (Cahill *et al.*,1993, FEMS Microbiology

Letters, 107, 211-216), most soluble antigens given intranasally by themselves induce little or no immune response.

A number of authors have investigated potential mucosal adjuvants to overcome this problem, which exert their adjuvant activity through various mechanisms including: encapsulation of the antigen (*e.g.* liposomes and microparticles); or *via* direct interaction with, and subsequent release of immunostimulatory cytokines from, target cells (*e.g.* cholera toxin and *E.coli* heat-labile toxin); or by enhancing the uptake of antigen across the epithelium (*e.g.* cholera toxin).

The applicant presents here the surprising finding that polyoxyethylene ethers and polyoxyethylene esters act as a potent mucosal adjuvants. The compositions of the present invention are safe, easily sterilisable, and simple to administer to a mucosal surface. Advantageously, such compositions are sufficient to induce systemic immune responses when administered mucosally, which are at least as high as those observed after conventional systemic injection of the vaccine.

Polyoxyethylene ethers such as polyoxyethylene lauryl ether are described in the Merck index (12th ed: entry 7717), where therapeutic uses are stated to include: topical anesthetic; anti-pruritic; and sclerosing agent activities. As a class, such polyoxyethylene ethers, or esters, are non-ionic surfactants.

Intranasal administration of poly(oxyethylene) ethers and esters have been described for the enhancement of insulin uptake in the nasal cavity (Hirai *et al.* 1981, International Journal of Pharmaceutics, 9, 165-172; Hirai *et al.* 1981, International Journal of Pharmaceutics, 9, 173-184).

Other non-ionic surfactants have been utilised in vaccine formulations. It has been reported that vaccine preparations comprising an admixture of either polyoxyethylene castor oil or caprylic/capric acid glycerides, with polyoxyethylene sorbitan monoesters, and an antigen, are capable of inducing systemic immune responses after topical administration to a mucosal membrane (WO 9417827). This

patent application discloses the combination of TWEEN20™ (polyoxyethylene sorbitan monoester) and Imwitor742™ (caprylic/capric acid glycerides), or a combination of TWEEN20™ and polyoxyethylene castor oil is able to enhance the systemic immune response following intranasal immunisation. Details of the effect of this formulation on the enhancement of the immune response towards intranasally administered antigens have also been described in the literature (Gizurarson *et al.* 1996. Vaccine Research, 5, 69-75; Aggerbeck *et al.* 1997. Vaccine, 15, 307-316).

Novasomes (US 5,147,725) are paucilamenar vesicular structures comprising Polyoxyethylene ethers and cholesterol which are capable of adjuvanting the immune response to antigens.

Surfactants have also been formulated in such a way as to form non-ionic surfactant vesicles (commonly known as neosomes, WO 95/09651). Such vesicles, in the presence of cholesterol form lipid-bilayer vesicles which are capable of entrapping antigen within the inner aqueous phase or within the bilayer itself.

We present here the surprising finding that relatively low concentrations of polyoxyethylene ethers or esters are able to significantly enhance the immune response towards intranasally administered antigens. Furthermore, the boosting effect of these adjuvants raises the systemic immunological responses to a level equal or superior to that achieved by conventional systemic injection of the antigen. These molecules, therefore, represent a class of mucosal adjuvants suitable for application in humans to replace systemic vaccination by intranasal vaccination.

The present invention comprises the surprising finding that polyoxyethylene ethers and esters are able to significantly enhance the systemic immune response when used as a vaccine adjuvant. The IgG titres induced by intranasal vaccination in the presence of polyoxyethylene ether were significantly superior to those induced by boosting in the absence of this surfactant. In addition, the LA2 titres, which are a measure of the bactericidal activity of the sera towards Borrelia burgdorferi show that the antibodies that are induced are of a bactericidal quality.

As many available vaccine adjuvants function because of antigen encapsulation, surprisingly the present invention functions as a potent mucosal adjuvant in the form of a non-vesicular solution or suspension. Thus, one embodiment of the present invention provides for an adjuvant formulation comprising a surfactant of formula (I), which is present in the form of a non-vesicular solution or suspension. Another embodiment of the present invention takes the form of a vaccine adjuvant comprising a surfactant of formula (I), formulated in the abscence of cholesterol.

It is foreseen that compositions of the present invention will be used to formulate vaccines containing antigens derived from a wide variety of sources. For example, antigens may include human, bacterial, or viral nucleic acid, pathogen derived antigen or antigenic preparations, tumour derived antigen or antigenic preparations, host-derived antigens, including the histamine releasing decapeptide of IgE (known as the Stanworth decapeptide), recombinantly produced protein or peptides, and chimeric fusion proteins.

The vaccine preparations of the present invention may be used to protect or treat a mammal susceptible to, or suffering from disease, by means of administering said vaccine via a mucosal route, such as the oral/alimentary or nasal route. Such administration may be in a droplet, spray, or dry powdered form. Nebulised or aerosolised vaccine formulations also form part of this invention.

The present invention provides for an adjuvant for use in mucosal vaccine formulations. Such adjuvants are well tolerated in humans and are potent in their induction of systemic immune responses. The adjuvants of the present invention may take the form of a solution, or non-vesicular solution or suspension, and as such do not have any of the problems associated with the manufacture, stability, uniformity, and quality control of particulate adjuvant systems.

The present applicants have demonstrated that the use of 1% polyoxyethylene ether induced an immune response against the antigen lipo-OspA which was of a

significantly greater magnitute than that observed after vaccination with a vaccine formulation comprising 36% TWEEN20™, or a mixture of 36% TWEEN20™ plus 10% Imwitor 742™.

Mixtures of other non-ionic surfactants have been reported to be reactogenic and cause significant local reactions. Agerbeck *et al.* describe the use of such TWEEN20™/Imwitor 742™ mixtures in human application. The application of the intranasal vaccines was reported to cause local symptoms including stinging and severe nasal secretions, in some cases causing severe reactions over a prolonged period. The reactogenicity was such that 40% of the vaccinees actually prefered the systemic needle based vaccination regime.

In contrast to this, published data demonstrating that daily use of polyoxyethylene ethers over a period of three months are acceptable to patients (Saltzman, R. et al., 1985, New England Journal of Medicine, 312, 1078-1084). This study showed that most patients tolerated daily administration of 1% polyoxyethylene ether (laureth 9), whilst all patients tolerated daily administration of 0.25% laureth 9.

Thus, in addition to the superior adjuvanticity activity of the polyoxyethylene ethers or esters, compared with a combination of 36% TWEEN20™ plus 10% Imwitor 742™, it is also an advantage of the present invention that reactogenicity in response to vaccination is reduced.

Vaccines and adjuvant formulations of the present invention comprise molecules of general formula (I):

$HO(CH_2CH_2O)_n$-A-R

wherein, n is 1-50, A is a bond or -C(O)-, R is C_{1-50} alkyl or Phenyl C_{1-50} alkyl.

One embodiment of the present invention consists of a vaccine formulation comprising a polyoxyethylene ether of general formula (I), wherein *n* is between 1 and 50, preferably 4-24, most preferably 9; the *R* component is C_{1-50}, preferably C_4-C_{20} alkyl and most preferably C_{12} alkyl, and *A* is a bond. The concentration of the

polyoxyethylene ethers should be in the range 0.1-20%, preferably from 0.1-10%, and most preferably in the range 0.1-1%.

A further embodiment of the present invention consists of a vaccine composition comprising a polyoxyethylene ester of general formula (I), wherein *n* is between 1 and 50, preferably 4-24, most preferably 9; *R* is C_{1-50}, preferably C_4 to C_{20} alkyl and most preferably C_{12} alkyl, and *A* is -C(O)-. The concentration of the polyoxyethylene ester should be in the range 0.1-20%, preferably from 0.1-10%, and most preferably in the range 0.1-1%.

Also forming an embodiment of the present invention are vaccine compositions comprising polyoxyethylene phenyl ethers of general formula (I), wherein *n* is between 1 and 50 but preferably 4-24 and most preferably 9, *R* is C_{1-50} phenyl alkyl, preferably C_4 - C_{20} phenyl alkyl, and most preferably C_{12} phenyl alkyl, and *A* is a bond. The concentration of the polyoxyethylene ethers should preferably be in the range 0.1-10%, and most preferably in the range 0.25-1%.

The ratio of the length of the polyoxyethylene section to the length of the alkyl chain in the surfactant (*i.e.* the ratio of n:m), affects the solubility of this class of detergent in an aqueous medium. Thus, the adjuvants of the present invention may be in solution or may form particulate structures such as micelles.

Vaccines of the present invention may take the form of a non-vesicular solution or suspension of polyoxyethylene ether or ester of general formula (I) in a pharmaceutically acceptable excipient, such as PBS or water, and an antigen or antigenic preparation. Such a vaccine formulation may then be applied to a mucosal surface of a mammal in either a priming or boosting vaccination regime.

Other adjuvants which are known to enhance both mucosal and systemic immunological responses include the bacterial enterotoxins derived from Vibrio Cholerae and Eschericia Coli (namely cholera toxin (CT), and heat-labile enterotoxin (LT) respectively). CT and LT are heterodimers consisting of a

pentameric ring of β-subunits, cradling a toxic A subunit. Their structure and biological activity are disclosed in Clements and Finklestein, 1979, Infection and Immunity, 24:760-769; Clements et al., 1980, Infection and Immunity, 24:91-97. Recently a non-toxic derivative of LT has been developed which lacks the proteolytic site required to enable the non-toxic form of LT to be "switched on" into its toxic form, once released from the cell. This form of LT (termed mLT(R192G)) is rendered insuceptible to proteolytic cleavage by a substitution of the amino acid arginine with glycine at position 192, and has been shown to have a greatly reduced toxicity whilst retaining its potent adjuvant activity. mLT(R192G) is, therefore, termed a proteolytic site mutant. Methods for the manufacture of mLT(R192G) are disclosed in the patent application WO 96/06627. Other mutant forms of LT include the active site mutants such as mLT(A69G) which contain a substitution of an glycine for an alanine in position 69 of the LTA sequence. The use of mLT(R192G) as a mucosal vaccine is described in patent application WO 96/06627. Such adjuvants may be advantageously combined with the non-ionic surfactants of the present invention.

Accordingly, in an alternative embodiment of the present invention the polyoxyethylene ether, or ester, will further be combined with other adjuvants or immunostimulants including Cholera toxin and its B subunit, Monophosphoryl Lipid A and its non-toxic derivative 3-de-O-acylated monophosphoryl lipid A (as described in UK patent no. GB 2,220,211), and the oligonucleotide adjuvant system CpG (as described in WO 96/02555, especially Krieg 1826). A Particularly prefered immunostimulant used in conjunction with POE is Krieg 1826 (also known herein as CpG 1001), which formulations are potent in the induction and boosting of immune responses in larger animals. Alternatively polyoxyethylene ethers or esters may be combined with vaccine vehicles composed of chitosan or other polycationic polymers, polylactide and polylactide-co-glycolide particles, particles composed of polysaccharides or chemically modified polysaccharides, liposomes and lipid-based particles, particles composed of glycerol monoesters, etc.

Vaccines of the present invention comprise the surfactants: polyoxyethylene ethers or esters and antigen. Thus the present invention includes the use of polyoxyethylene ethers and esters of general formula (I) in the manufacture of adjuvant compositions or vaccines, wherein the surfactant of general formula (I) is not present in a vesicular form.

Preferably the vaccine formulations of the present invention contain an antigen or antigenic composition capable of eliciting an immune response against a human pathogen, which antigen or antigenic composition is derived from HIV-1, (such as tat, nef, gp120 or gp160), human herpes viruses, such as gD or derivatives thereof or Immediate Early protein such as ICP27 from HSV1 or HSV2, cytomegalovirus ((esp Human)(such as gB or derivatives thereof), Rotavirus (including live-attenuated viruses), Epstein Barr virus (such as gp350 or derivatives thereof), Varicella Zoster Virus (such as gpI, II and IE63), or from a hepatitis virus such as hepatitis B virus (for example Hepatitis B Surface antigen or a derivative thereof), hepatitis A virus, hepatitis C virus and hepatitis E virus, or from other viral pathogens, such as paramyxoviruses: Respiratory Syncytial virus (such as F and G proteins or derivatives thereof), parainfluenza virus, measles virus, mumps virus, human papilloma viruses (for example HPV6, 11, 16, 18, ..), flaviviruses (e.g. Yellow Fever Virus, Dengue Virus, Tick-borne encephalitis virus, Japanese Encephalitis Virus) or Influenza virus, or derived from bacterial pathogens such as *Neisseria spp,* including *N. gonorrhea* and *N. meningitidis* (for example capsular polysaccharides and conjugates thereof, transferrin-binding proteins, lactoferrin binding proteins, PilC, adhesins); *Streptococcus spp,* including *S. pneumoniae* (for example capsular polysaccharides and conjugates thereof, PsaA, PspA, streptolysin, choline-binding proteins), *S. pyogenes* (for example M proteins or fragments thereof, C5A protease, lipoteichoic acids), *S. agalactiae, S. mutans; Haemophilus spp, including H. influenzae type B* (for example PRP and conjugates thereof), *non typeable H. influenzae* (for example OMP26, high molecular weight adhesins, P5, P6, lipoprotein D), *H. ducreyi; Moraxella spp, including M catarrhalis, also known as Branhamella* catarrhalis (for example high and low molecular weight adhesins and invasins*); Bordetella spp, including B. pertussis* (for example pertactin,

pertussis toxin or derivatives thereof, filamenteous hemagglutinin, adenylate cyclase, fimbriae), *B. parapertussis and B. bronchiseptica; Mycobacterium spp., including M. tuberculosis* (for example ESAT6, Antigen 85A, -B or -C), *M. bovis, M. leprae, M. avium, M. paratuberculosis, M. smegmatis; Legionella spp,* including *L. pneumophila; Escherichia spp,* including *enterotoxic E. coli* (for example colonization factors, heat-labile toxin or derivatives thereof, heat-stable toxin or derivatives thereof), enterohemorragic *E. coli,* enteropathogenic *E. coli* (for example shiga toxin-like toxin or derivatives thereof); *Vibrio spp,* including *V. cholera* (for example cholera toxin or derivatives thereof); *Shigella spp,* including *S. sonnei, S. dysenteriae, S. flexnerii; Yersinia spp,* including *Y.* enterocolitica (for example a Yop protein) , *Y. pestis, Y. pseudotuberculosis; Campylobacter spp,* including *C. jejuni* (for example toxins, adhesins and invasins) and *C. coli; Salmonella spp,* including *S. typhi, S. paratyphi, S. choleraesuis, S. enteritidis; Listeria spp.,* including *L. monocytogenes; Helicobacter spp,* including *H.* pylori (for example urease, catalase, vacuolating toxin); *Pseudomonas spp,* including *P. aeruginosa; Staphylococcus spp.,* including *S. aureus, S. epidermidis; Enterococcus spp.,* including *E. faecalis, E. faecium; Clostridium spp.,* including *C.* tetani (for example tetanus toxin and derivative thereof), *C. botulinum* (for example botulinum toxin and derivative thereof), *C. difficile* (for example clostridium toxins A or B and derivatives thereof); *Bacillus spp.,* including *B. anthracis* (for example botulinum toxin and derivatives thereof); *Corynebacterium spp.,* including *C. diphtheriae* (for example diphtheria toxin and derivatives thereof); *Borrelia spp.,* including *B. burgdorferi* (for example OspA, OspC, DbpA, DbpB), *B. garinii* (for example OspA, OspC, DbpA, DbpB), *B. afzelii* (for example OspA, OspC, DbpA, DbpB), *B. andersonii* (for example OspA, OspC, DbpA, DbpB), *B. hermsii; Ehrlichia spp.,* including *E. equi* and the agent of the Human Granulocytic Ehrlichiosis; *Rickettsia spp,* including *R. rickettsii; Chlamydia spp., including C. trachomatis* (for example MOMP, heparin-binding proteins), *C. pneumoniae* (for example MOMP, heparin-binding proteins), *C. psittaci; Leptospira spp.,* including *L. interrogans; Treponema spp.,* including *T. pallidum* (for example the rare outer membrane proteins), *T. denticola, T. hyodysenteriae;* or derived from parasites such as *Plasmodium spp.,* including *P. falciparum; Toxoplasma spp.,* including *T. gondii (for example SAG2,*

SAG3, Tg34); Entamoeba spp., including *E. histolytica; Babesia spp.*, including *B. microti; Trypanosoma spp.*, including *T. cruzi; Giardia spp.*, including *G. lamblia; Leshmania spp.*, including *L. major; Pneumocystis spp.*, including *P. carinii; Trichomonas spp.*, including *T. vaginalis; Schisostoma spp.*, including *S. mansoni,* or derived from yeast such as *Candida spp.*, including *C. albicans; Cryptococcus spp.*, including *C. neoformans.*

Derivatives of Hepatitis B Surface antigen are well known in the art and include, inter alia, those PreS1, PreS2 S antigens set forth described in European Patent applications EP-A-414 374; EP-A-0304 578, and EP 198-474. In one preferred aspect the vaccine formulation of the invention comprises the HIV-1 antigen, gp120, especially when expressed in CHO cells. In a further embodiment, the vaccine formulation of the invention comprises gD2t as hereinabove defined.

In a preferred embodiment of the present invention vaccines containing the claimed adjuvant comprise the HPV viruses considered to be responsible for genital warts, (HPV 6 or HPV 11 and others), and the HPV viruses responsible for cervical cancer (HPV16, HPV18 and others). Particularly preferred forms of vaccine comprise L1 particles or capsomers, and fusion proteins comprising one or more antigens selected from the HPV 6 and HPV 11 proteins E6, E7, L1, and L2. The most preferred forms of fusion protein are: L2E7 as disclosed in GB 95 15478.7, and proteinD(1/3)-E7 disclosed in GB 9717953.5.

Vaccines of the present invention further comprise antigens derived from parasites that cause Malaria. For example, preferred antigens from *Plasmodia falciparum* include RTS,S and TRAP. RTS is a hybrid protein comprising substantially all the C-terminal portion of the circumsporozoite (CS) protein of *P.falciparum* linked via four amino acids of the preS2 portion of Hepatitis B surface antigen to the surface (S) antigen of hepatitis B virus. It's full structure is disclosed in the International Patent Application No. PCT/EP92/02591, published under Number WO 93/10152 claiming priority from UK patent application No.9124390.7. When expressed in yeast RTS is produced as a lipoprotein particle, and when it is co-expressed with the

S antigen from HBV it produces a mixed particle known as RTS,S. TRAP antigens are described in the International Patent Application No. PCT/GB89/00895, published under WO 90/01496. A preferred embodiment of the present invention is a Malaria vaccine wherein the antigenic preparation comprises a combination of the RTS,S and TRAP antigens. Other plasmodia antigens that are likely candidates to be components of a multistage Malaria vaccine are *P. faciparum* MSP1, AMA1, MSP3, EBA, GLURP, RAP1, RAP2, Sequestrin, PfEMP1, Pf332, LSA1, LSA3, STARP, SALSA, PfEXP1, Pfs25, Pfs28, PFS27/25, Pfs16, Pfs48/45, Pfs230 and their analogues in Plasmodium spp.

The formulations may also contain an anti-tumour antigen and be useful for the immunotherapeutic treatment cancers. For example, the adjuvant formulation finds utility with tumour rejection antigens such as those for prostrate, breast, colorectal, lung, pancreatic, renal or melanoma cancers. Exemplary antigens include MAGE 1 and MAGE 3 or other MAGE antigens for the treatment of melanoma, PRAME, BAGE or GAGE (Robbins and Kawakami, 1996, Current Opinions in Immunology 8, pps 628-636; Van den Eynde et al., International Journal of Clinical & Laboratory Research (submitted 1997); Correale et al. (1997), Journal of the National Cancer Institute 89, p293. Indeed these antigens are expressed in a wide range of tumour types such as melanoma, lung carcinoma, sarcoma and bladder carcinoma. Other Tumor-Specific antigens are suitable for use with adjuvant of the present invention and include, but are not restricted to Prostate specific antigen (PSA) or Her-2/neu, KSA (GA733), MUC-1 and carcinoembryonic antigen (CEA). Accordingly in one aspect of the present invention there is provided a vaccine comprising an adjuvant composition according to the invention and a tumour rejection antigen.

It is foreseen that compositions of the present invention will be used to formulate vaccines containing antigens derived from *Borrelia sp.*. For example, antigens may include nucleic acid, pathogen derived antigen or antigenic preparations, recombinantly produced protein or peptides, and chimeric fusion proteins. In particular the antigen is OspA. The OspA may be a full mature protein in a lipidated

form virtue of the host cell (E.Coli) termed (Lipo-OspA) or a non-lipidated derivative. Such non-lipidated derivatives include the non-lipidated NS1-OspA fusion protein which has the first 81 N-terminal amino acids of the non-structural protein (NS1) of the influenza virus, and the complete OspA protein, and another, MDP-OspA is a non-lipidated form of OspA carrying 3 additional N-terminal amino acids.

Vaccines of the present invention may be used for the prophylaxis or tharapy of allergy. Such vaccines would comprise allergen specific (for example Der p1) and allergen non-specific antigens (for example the stanworth decapeptide).

The amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed and how it is presented. Generally, it is expected that each dose will comprise 1-1000 mg of protein, preferably 1-500 mg, preferably 1-100μg, most preferably 1 to 50μg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in subjects. Following an initial vaccination, subjects may receive one or several booster immunisation adequately spaced.

The present invention includes a method of producing a vaccine or adjuvant composition comprising admixing a surfactant of general formula (I) and a pharmaceuticaly acceptable excipient, and in the case of a vaccine, an antigen or antigenic composition.

Alternative terms or names for polyoxyethylene lauryl ether are disclosed in the CAS registry. The CAS registry number of polyoxyethylene lauryl ether is:
CAS REGISTRY NUMBER: ***9002-92-0***

Examples of suitable pharmaceutically acceptable excipients include water, phosphate buffered saline, isotonic buffer solutions. The vaccines of the present

invention may also be administered via the oral route. In such cases the pharmaceutically acceptible excipient may also include alkaline buffers, or digestible capsules.

The formulations of the present invention maybe used for both prophylactic and therapeutic purposes. Accordingly, the present invention provides for a method of treating a mammal susceptible to or suffering from an infectious disease or cancer, or allergy, or autoimmune disease. In a further aspect of the present invention there is provided a vaccine as herein described for use in medicine. Vaccine preparation is generally described in New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978.

The present invention relates to the use of polyoxyethylene ethers or esters of general formula (I) in the manufacture of an adjuvant formulation, comprising a surfactant of formula (I) and a pharmaceutically acceptable excipient. The present invention relates to the use of polyoxyethylene ethers or esters of general formula (I) in the manufacture of vaccine formulation, comprising a surfactant of formula (I) and a pharmaceutically acceptable excipient and an antigen. The present invention also relates to the use of polyoxyethylene ethers or esters of general formula (I) in the manufacture of an adjuvant formulation or vaccine, as described above, wherein the formulation does not contain cholesterol. The present invention further provides the use of polyoxyethylene ethers or esters of general formula (I) in the manufacture of an adjuvant formulation or vaccine, as described above, wherein the formulation is a non-vesicular solution or suspension.

The present invention is illustrated by, but not restricted to, the following examples.

Example 1, *Techniques used to measure anti-OspA antibody responses.*

ELISA for the measurement of OspA-specific serum IgG:

Maxisorp Nunc immunoplates are coated overnight at 4°C with 50 µl/well of 1 µg/ml OspA diluted in PBS (in rows B to H of plate), or with 50 µl of 5 µg/ml

purified goat anti-mouse Ig (Boerhinger), in PBS (row A). Free sites on the plates were blocked (1 hour, 37°C) using saturation buffer : PBS comtaining 1%BSA, 0.1% polyoxyethylene sorbitan monolaurate (TWEEN 20), and 4% Normal Bovine Serum (NBS). Then, serial 2-fold dilutions of IgG isotype mixture, diluted in saturation buffer (50 µl per well), was added as a standard curve (mixture of mouse monoclonal antibodies IgG1, IgG2a and IgG2b from Sigma, starting at 200 ng/ml and put in row A) and serum samples (starting at a 1/100 dilution and put in rows B to H) are incubated for 1hr 30mins at 37°C. The plates were then washed (×3) with washing buffer (PBS, 0.1% polyoxyethylene sorbitan monolaurate (TWEEN 20)). Then, biotinylated goat anti-mouse IgG (Amersham) diluted 1/5000 in saturation buffer are incubated (50 µl/well) for 1hr 30mins, at 37°C. After 3 washings, and subsequent addition of streptavidin-horseradish peroxidase conjugate (Amersham), plates are washed 5 times and incubated for 20 min at room temperature with 50 µl/well of revelation buffer (OPDA 0.4 mg/ml (Sigma) and H_2O_2 0.03% in 50mM pH 4.5 citrate buffer). Revelation is stopped by adding 50 µl/well H_2SO_4 2N. Optical densities are read at 492 and 630 nm by using Biorad 3550 immunoreader. Antibody titre are calculated by the 4 parameter mathematical method using SoftMaxPro software.

Inhibition assay for the measurement of serum LA2-like Antibody titres to lipo-OspA

Antibody titres in the vaccinees were studied with respect to their LA2-like specificity. LA2 is a murine monoclonal antibody which recognizes a conformational OspA epitope at the surface of the bacteria and has been shown to be able to kill B. burgdorferi in vitro, as well as to protect mice against a challenge with laboratory-grown spirochete (Schaible UE et al. 1990. Proc Natl Acad Sci USA 87:3768-3772). Moreover, LA-2 mab has been shown to correlate with bactericidal antibodies, and studies on human sera showed also a good correlation between the total anti-OspA IgG titers and the LA-2 titers (as measured by ELISA).

Maxisorp Nunc immunoplates are coated overnight at 4°C with 50 µl/well of 0.5µg/ml lipo OspA diluted in PBS. Free sites were blocked with saturation buffer

for 1hr at 37°C with (100 µl/well of saturation buffer: PBS/ BSA 1%/ Tween 20 0.1%/ NBS 4%). Serial 2-fold dilutions of LA2 monoclonal Ab (mAb) starting at 4 µg/ml were diluted in saturation buffer (50 µl per well) to form a standard curve. Dilutions of serum samples from the vaccinees (starting at a 1/10 dilution) were also added and the plates incubated for 2hrs at 37°C. The plates were washed after incubation 3 times with PBS/ TWEEN 20 (0.1%). LA2 mAb-peroxidase conjugate (1/10,000) diluted in saturation buffer was added to each well (50 µl/well) and incubated for 1hr at 37°C. After 5 washings, plates are incubated for 20 min at room temperature (in darkness) with 50 µl/well of revelation buffer (OPDA 0.4 mg/ml and H_2O_2 0.03% in 50mM pH 4.5 citrate buffer). The reaction and colour formation was stopped with H_2SO_4 2N. Optical densities are read at 492 and 630 nm by using Biorad 3550 immunoreader. LA2-like Ab titers are calculated by the 4 parameter mathematical method using SoftMaxPro software. LA2-like antibody titres were determined by comparison with the standard curve.

Example 2

Female Balb/c mice (8 animals per group) aged 8 weeks were immunised intramuscularly with 1 µg of the antigen lipo-OspA on 50 µg alum. After 3 months the mice were boosted intranasally (under anesthesia) with 10 µl of solution (5 µl per nostril, delivered as droplets by pipette) containing either A: 5 µg lipo-OspA; B: 5 µg lipo-OspA in 36 % tween-20, 10% Imwitor 742 ; C: 5 µg lipo-OspA in 36 % tween-20; D: 5 µg lipo-OspA in 18% polyoxyethylene-9 lauryl ether.
14 days after the boost the sera were assayed for IgG against lipo-OspA by anti-OspA and anti-LA2 ELISA (see example 1). The results, see figures 1 and 2, indicate that lipo-OspA administered intranasally is able to boost the systemic lipo-OspA specific IgG titres. This boost is only marginally increased by the presence of tween-20 plus Imwitor 742 or tween-20 alone. Polyoxyethylene-9 lauryl ether, on the other hand, induces a very significant boost. A similar pattern is observed for the LA2 response.

Example 3

Groups of mice were primed as described in example 2. The mice were then boosted (using the method described in example 2) with 5 μg lipo-OspA alone (group A and C) or in the presence of B: 1 % sodium taurocholic acid; D: 1% dodecyl-maltoside; E: 36% tween 20 or F: 18% polyoxyethylene-9 lauryl ether. Since the experiment with groups A and B was performed at a different moment to that with groups C,D,E and F they are separated on the figures below (see figure 3). It is clear that 1% sodium taurocholate does not significantly adjuvant the boost above that obtained with the antigen alone. Dodecyl-maltoside at 1%, or tween-20 at 36% provide a slight adjuvant effect, but only polyoxyethylene-9 lauryl ether provides a very significant enhancement of the IgG response. A similar effect is observed for the LA2 response (see figure 4).

Example 4

In order to asses the concentration of polyoxyethylene-9 lauryl ether required to provide the nasal adjuvanticity observed in the previous examples, we performed a dose-range assay, and in order to show that this effect can be achieved using other polyoxyethylene ethers we investigated the use of polyoxyethylene-20 lauryl ether. Mice primed as in example 1 were boosted intranasally with 10 μl containing 5 μg of lipo-OspA in either A: PBS; B: 1% polyoxyethylene-9 lauryl ether; C: 2% polyoxyethylene-9 lauryl ether; D: 5% polyoxyethylene-9 lauryl ether; E: 1% polyoxyethylene-20 lauryl ether or; F: 10 % polyoxyethylene-20 lauryl ether. 14 days after the boost the sera were analysed as in example 1.

Figures 5 and 6, below, show that concentrations of polyoxyethylene-9 lauryl ether as low as 1% show a very significant enhancement of the immune response. Polyoxyethylene-20-lauryl ether also significantly enhances the intranasal boost response.

Example 5

In order to asses the applicability of polyoxyethylene ethers to the enhancement of systemic immune responses after intranasal boosting, female balb/c mice were primed intra-muscularly with the commercial DTPa vaccine (Diptheria, Tetanus, accelular Pertussis vaccine: INFANRIX™ SmithKline Beecham, Belgium). The mice

were primed once intramuscularly with 2 X 50 μl injections corresponding to 20% of the human dose. Three months later the mice were boosted (as in example 1) intranasally with either tetanus toxoid (TT: 5 μg) or filamentous haemagglutinin (FHA: 5 μg) in A: PBS; B: 1% polyoxyethylene-9 lauryl ether; or; C: by intramuscular injection of the DTPa vaccine (2×50μg). 14 days after the boosting the sera were analysed for their TT and FHA specific IgG. The titres are shown in figures 7 and 8.

It is clear that for TT the protein by itself does not induce a significant boost, but polyoxyethylene-9 lauryl ether is able to significantly boost the immune response. Surprisingly, the response obtained by intranasal boosting in the presence of this adjuvants is greater than that obtained following intramuscular boosting of the immune response.The administration of FHA by itself, induces an immune response which is further significantly enhanced by addition of the polyoxyethylene-9 lauryl ether as an adjuvant.

Example 6

Many adjuvants have been shown to work in small rodents, but to have no effect when tested in larger mammals. In order to asses whether polyoxyethylene ethers were able to exert an adjuvant effect on intranasal boosting when this was performed in larger species, African Green monkeys (AGMs: 4 animals per group) were primed intramuscularly with lipo-OspA (10 μg) on alum (500 μg) by intramuscular injection. 10 months later the animals were boosted intranasally with 200 μl (100 μl per nostril administered under anesthesia with a bidose spray device from Pfeiffer GmBH, Germany) containing 60 μg lipo-OspA in either A: PBS; or B: 1% polyoxyethylene-9 lauryl ether. After 14 days the sera were tested for anti-OspA immunoglobulin, and LA2 titres.Figures 9 and 10, show the geometric mean titres titres for each of the groups. Group C consisting of 10 AGMs that had received both the priming and the boost by intramuscular injection of lipo-OspA on alum were assayed for anti-OspA immunoglobulin responses (geometric mean titres shown for LA2 titres only, figure 10).

Lipo-OspA alone was able to boost the systemic response when administered intranasally to monkeys, but this boost is very significantly enhanced by the addition of 1% polyoxyethylene 9 lauryl ether. Surprisingly, the titres obtained following intranasal boosting in the presence of polyoxyethylene 9 lauryl ether are also greater than those obtained following an intramuscular injection (group C).

Example 7

In the previous examples we demonstrated that polyoxyethylene ethers could adjuvant an intranasal boosting of the systemic response. In this example we examine whether naive animals can be primed and boosted by the nasal route to induce a systemic immune response. In addition, in order to investigate the applicability of these adjuvants to larger animals, this experiment was performed in African Green Monkeys (AGMs).

African Green Monkeys (3 animals per group) were primed and boosted intranasally with 60 μg of lipo-OspA delivered in 200 μl (100 μl per nostril delivered with a bidose spray-device from Pfeiffer GmBH, Germany) of A: PBS; B: 1% polyoxyethylene-9 lauryl ether. 14 days after the boosting the sera were assayed for their Osp-A specific immunoglobulin. Figure 11, shows that when Lipo-OspA is not adjuvanted, no systemic immune response can be detected following intranasal priming and boosting. When polyoxyethylene-9 lauryl ether is used as an adjuvant, this vaccination schedule induced significant anti-OspA titres.

Example 8, *Intranasal adjuvant effect of CpG on the induction of systemic and nasal humoral immune responses to lipo OspA antigen in primates*

This model was designed to investigate the priming and boosting effect of polyoxyethylene lauryl ether, with and without additional immunostimulants, in a primate priming and boosting model. Serum and nasal immunoglobulin responses were measured. The immunostimulant used in this study was the CpG 1001 as described in example 9.

Experimental procedure

African Green monkeys were primed and boosted intranasally at days 0 (pI) and 14 (pII). Vaccines were given using a bi-dose spray delivery system from the Pfeiffer company (100 µl in each nostril, under anesthesia). Formulations tested were:

Group	***Antigen***	***Adjuvant***	***n=***	***Route***
1	LipoOspA (60µg)	None	2	i.n.
2	lipoOspA (60µg)	CpG (100µg)	3	i.n.
3	lipoOspA (60µg)	CpG (100µg), POE (0.25%)	3	i.n.
4	lipoOspA (60µg)	POE (0.25%)	4	i.n.
5	lipoOspA (60µg)	POE (0.5%)	4	i.n.

Ig Ab titers to lipo OspA were measured in sera collected at day 14 post-pII. Antigen-specific nasal IgA were measured using a very sensitive ELISA in nasal swabs collected at the same time, animals were considered positive when their IgA titres exceeded a pre-determined level which was significantly above background levels.

Results

Serum OspA-specific immunoglobulin

Figure 12 shows the levels of serum anti-lipo-OspA immunoglobulin responses observed at day 14 post-pII. Lipo-OspA given as a priming and boosting formulation alone did not induce any detectable serum immunoglobulin. This response was not improved in the presence of CpG. A dose of 0.25 % and 0.5 % of polyoxyethylene lauryl ether elicited greater immune responses that those observed after vaccination with CpG alone, although the 0.5% dose is much more efficient in this respect. However, when combined with CpG, the 0.25 % dose induces an Ab response similar in magnitude to that obtained with 0.5 % dose, indicating a synergistic effect of the CpG and POE components.

Nasal OspA-specific IgA

As observed for the serum Ig response, vaccines containing lipo OspA alone or combined with CpG are unable to elicit detectable nasal IgA Abs (see figure 13 for a summary of all nasal responses). Only 25% animals given lipo OspA in combination with 0.25% polyoxyethylene lauryl ether were found to be "nasal IgA" positive (versus 50% in the 0.5% polydocanol group). When CpG is added to this 0.25% POE formulation, 100% animals develop an IgA response. Therefore, a synergy between CpG and polyoxyethylene lauryl ether is also obtained for the induction of mucosal antibodies.

Thus, a synergy between polyoxyethylene lauryl ether detergent and CpG is obtained in monkeys for the induction of antigen specific serum immunoglobulins and nasal IgA.

Example 9, *Intranasal adjuvant effect of CpG on the boosting of systemic humoral immune responses to lipo OspA antigen*

The following example was designed to investigate the effect of the addition of other immunostimulants into the polyoxyethylene ether (POE) adjuvant system in a murie booster model. CpG is a known immunomodulatory oligonucleotide described in PCT WO 96/02555. The immune response boosted by these vaccine formulations were at least as high as those induced by conventional i.m. boosting vaccinations. The formulations were further compared to a well known intranasal adjuvant, the heat-labile enterotoxin from *E.Coli* (mLT).

The CpG sequences used in this experiment were CpG 1001 (TCC ATG AGC TTC CTG ACG TT, Krieg 1826), CpG 1002 (TCT CCC AGC GTG CGC CAT, Krieg 1758), and the negative control the non-immunostimulatory sequence CpG1005 (TCC ATG AGC TTC CTG AGC TT, Krieg 1826).

Experimental procedure

Balb/c mice were primed at day 0 (pI) by intramuscular administration of 100 µl vaccine containing 1 µg lipo OspA adsorbed on 50 µg aluminium hydroxyde (1/10 human dose). At day 107, intranasal booster was given in 10 µl (5 µl in each nostril), by nasal drop administration with a micropipette under anesthesia. Groups of 6 mice were boosted either intranasally (i.n.) or intramuscularly (i.m.) with the following vaccine formulations:

Group	***Antigen***	***Adjuvant***	***Route***
1	LipoOspA (5µg)	$AlOH_3$ (50µg)	i.m.
2	LipoOspA (5µg)	CpG1005 (20µg), POE (1%)	i.n.
3	LipoOspA (5µg)	CpG1002 (20µg), POE (1%)	i.n.
4	LipoOspA (5µg)	CpG1001 (20µg), POE (1%)	i.n.
5	LipoOspA (5µg)	CpG1005 (20µg)	i.n.
6	LipoOspA (5µg)	CpG1002 (20µg)	i.n.
7	LipoOspA (5µg)	CpG1001 (20µg)	i.n.
8	LipoOspA (5µg)	POE (1%)	i.n.
9	LipoOspA (5µg)	mLT (5µg)	i.n.
10	LipoOspA (5µg)	None	i.n.
11	Unboosted		

Bleedings were performed the day of boosting, and 14 days after the boost (pII). Specific serum IgG titers to OspA and LA2 titers were determined by ELISA on individual sera.

Results

As shown in figure 14 (showing OspA specific serum IgG as measured by antigen specific ELISA), and figure 15 (showing bacteriocidal anti-LA2 titres in serum), no improvement of the serum OspA-specific Ab responses was imparted by CpG alone. The formulation of OspA with polyoxyethylene lauryl ether enhanced the resultant IgG and LA2 titers. The best responses were observed when lipo-OspA was

formulated with both polyoxyethylene lauryl ether and CpG. However, these increased responses are not considered to be statistically significant.

Claims:

1. A vaccine composition comprising polyoxyethylene ether or a polyoxyethylene ester, in combination with a pharmaceutically acceptable excipient, and an antigen or antigenic composition, wherein the polyoxyethylene ether or ester is not in the form of a vesicle.

2. A vaccine composition comprising a surfactant of formula (I):

$HO(CH_2CH_2O)_n$-A-R

wherein, n is 1-50, A is a bond or -C(O)-, R is C_{1-50} alkyl or Phenyl C_{1-50} alkyl; a pharmaceutically acceptable excipient, and an antigen or antigenic composition, wherein the surfactant is not in the form of a vesicle.

3. A vaccine composition as claimed in claim 2, comprising a surfactant of formula (I), wherein n is 4-24.

4. A vaccine composition as claimed in claim 2, comprising a surfactant of formula (I), wherein n is 9.

5. A vaccine composition as claimed in any one of claims 2 to 4, comprising a surfactant of formula (I), wherein R is C_{8-20} alkyl or Phenyl C_{8-20} alkyl.

6. A vaccine composition as claimed in any one of claims 2 to 4, comprising a surfactant of formula (I), wherein R is C_{12} alkyl or Phenyl C_{12} alkyl.

7. A vaccine composition as claimed in any one of claims 2 to 6, comprising a surfactant of formula (I), wherein A is a bond, thereby forming an ether.

8. A vaccine composition as claimed in any one of claims 2 to 6, comprising a surfactant of formula (I), wherein A is -C(O)-, thereby forming an ester.

9. A vaccine composition as claimed in claim 1, comprising a polyoxyethylene ether or ester, selected from polyoxyethylene-9-lauryl ether, polyoxyethylene-9-lauryl ester, polyoxyethylene-9-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether.

10. A vaccine composition as claimed in claim 2, comprising a surfactant selected from polyoxyethylene-9-lauryl ether, polyoxyethylene-9-lauryl ester, polyoxyethylene-9-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether.

11. A vaccine composition as claimed in any one of claims 1 to 10, wherein the concentration of the surfactant is in the range 0.1-10%.

12. A vaccine composition as claimed in any one of claims 1 to 10, wherein the concentration of the surfactant is in the range 0.25-1%.

13. A vaccine composition as claimed in any one of claims 1 to 12, wherein the antigen or antigen composition is derived from the group comprising: Human Immunodeficiency Virus, Varicella Zoster virus, Herpes Simplex Virus type 1, Herpes Simplex virus type 2, Human cytomegalovirus, Dengue virus, Hepatitis A, B, C or E, Respiratory Syncytial virus, human papilloma virus, Influenza virus, Hib, Meningitis virus, Salmonella, Neisseria, Borrelia, Chlamydia, Bordetella, Streptococcus, Haemophilus, Plasmodium or Toxoplasma, stanworth decapeptide; or Tumor associated antigens (TMA), MAGE, BAGE, GAGE, MUC-1, Her-2 neu, LnRH, CEA, PSA, KSA, or PRAME.

14. A vaccine composition as claimed in any one of claim 1 to 13, further comprising other adjuvants.

15. A vaccine composition as claimed in any one of claim 1 to 13, further comprising other adjuvants selected from the group comprising: LT, CT, MPL, CpG, QS21.

16. A vaccine composition as claimed in claim 15, wherein the CpG adjuvant is Krieg 1826.

17. A vaccine composition as claimed in any one of claim 1 to 16, further comprising a vehicle, said vehicle comprising of any one of the following group: chitosan or other polycationic polymers, polylactide and polylactide-co-glycolide particles, particles composed of polysaccharides or chemically modified polysaccharides, or particles composed of glycerol monoesters.

18. Use of a polyoxyethylene ether or ester, in the manufacture of an adjuvant composition, wherein the polyoxyethylene ether or ester is present in the adjuvant composition in a non-vesicular form.

19. Use of a surfactant of general formula (I), in the manufacture of an adjuvant composition, wherein the surfactant of general formula (I) is present in the adjuvant composition in a non-vesicular form.

20. Use of vaccine composition as defined in any of claims 1 to 17, for the manufacture of a vaccine for the treatment of viral, bacterial, parasitic infections, allergy, or cancer.

21. A method of treating a mammal suffering from or susceptible to a pathogenic infection, or cancer, or allergy, comprising the administration of a safe and effective amount of a composition according to any of claims 1 to 17.

22. A method of treating a mammal suffering from or susceptible to a pathogenic infection, or cancer, or allergy, comprising the mucosal administration of a safe and effective amount of a composition according to any of claims 1 to 17.

23. A method of treating a mammal suffering from or susceptible to a pathogenic infection, or cancer, or allergy, comprising the intranasal administration of a safe and effective amount of a composition according to any of claims 1 to 17.

24. A process for making a vaccine composition according to claim 1, comprising admixing a polyoxyethylene ether or ester, a pharmaceutically acceptable excipient, and an antigen or antigenic composition.

25. A process for making a vaccine composition as claimed in any one of claims 2 to 17, comprising admixing a surfactant of general formula (I), a pharmaceutically acceptable excipient, and an antigen or antigenic composition.

Figure 1, Anti-OspA responses in mice (see example 2)

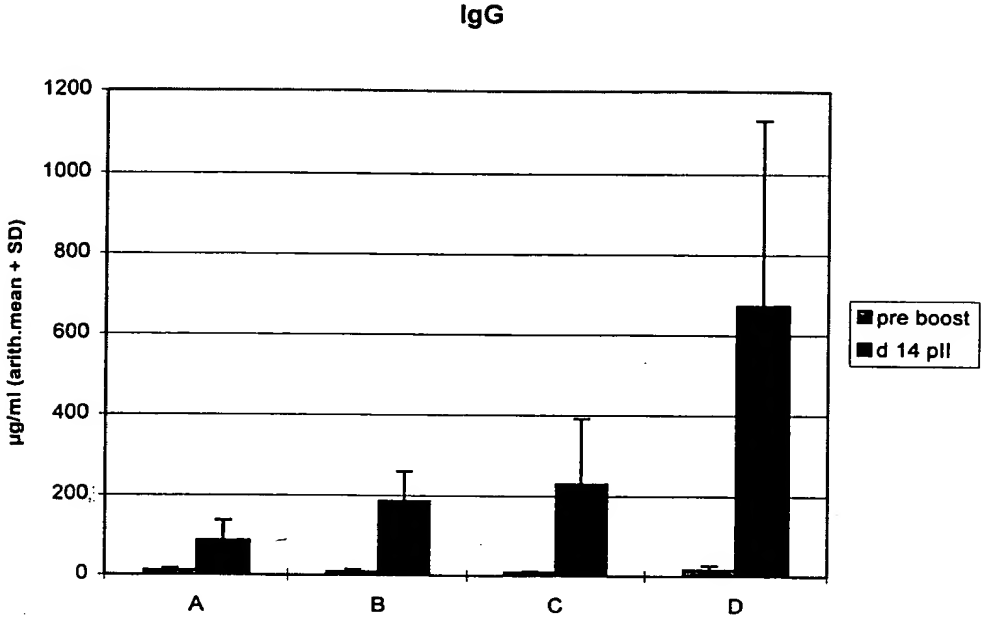

Figure 2, Anti-LA2 titres in mice (see example 2)

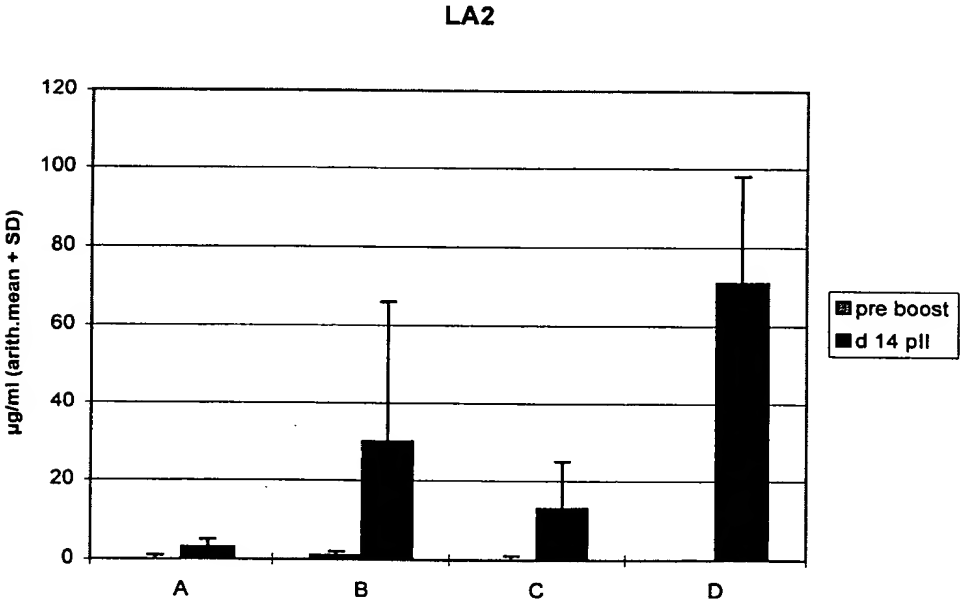

Figure 3, Anti-OspA responses in mice (see example 3).

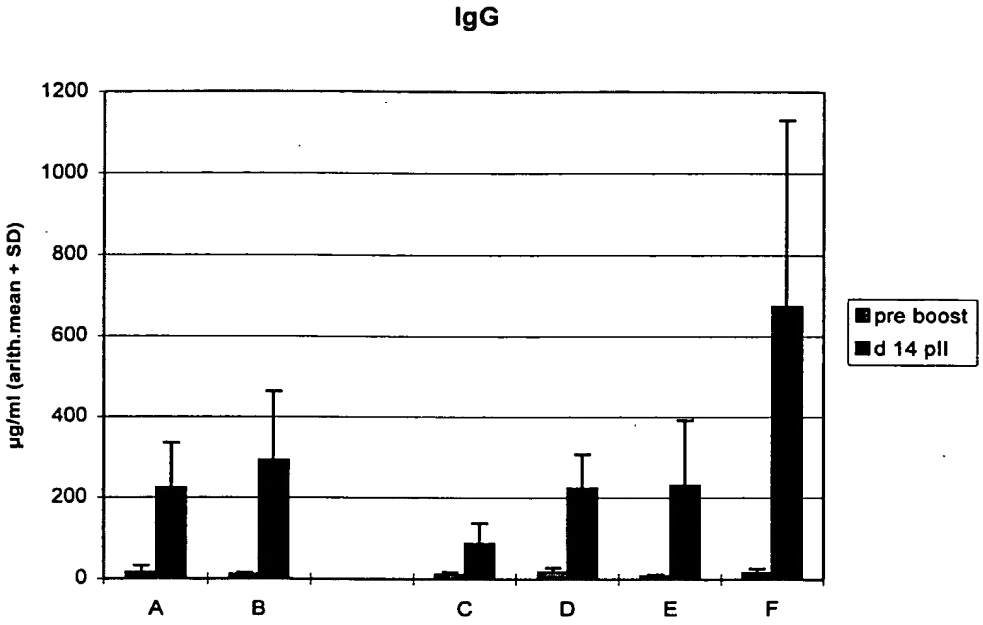

Figure 4, Anti-LA2 titres in mice (see example 3)

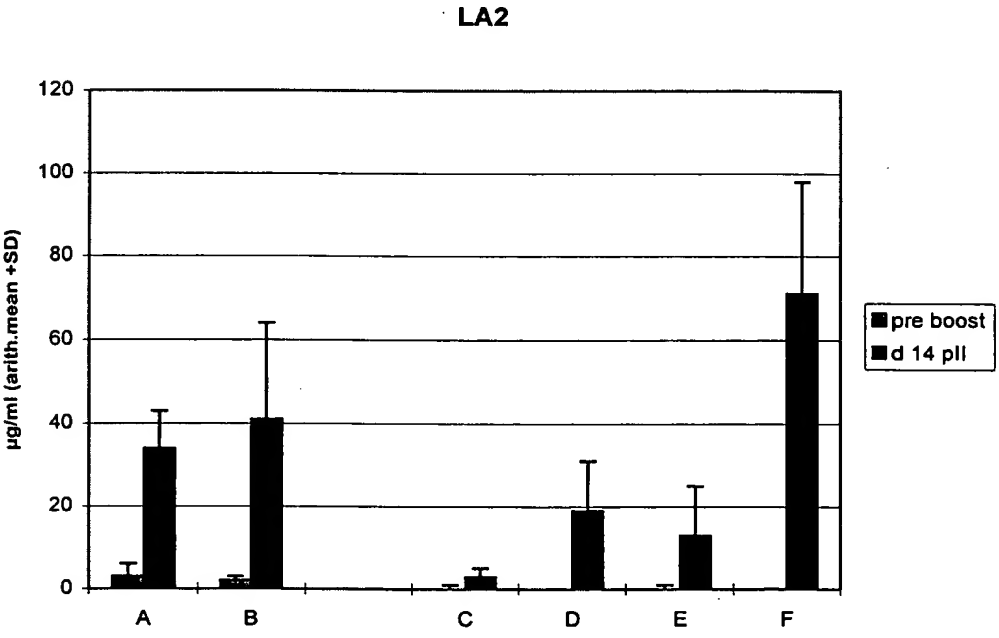

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Figure 5, Anti-OspA antibody titres (see example 4)

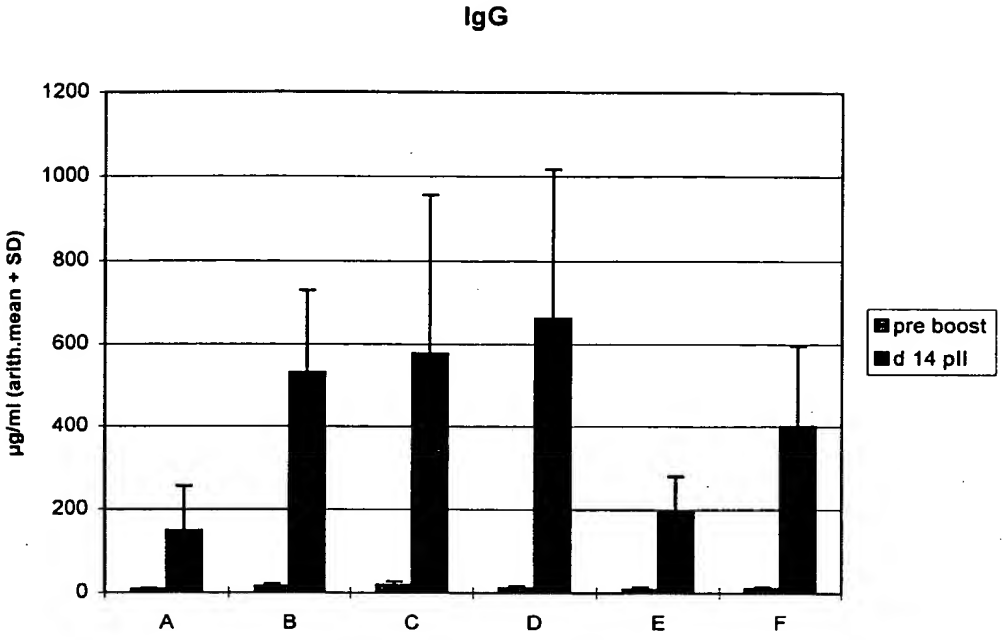

Figure 6, anti-LA2 titres in mice (see xample 4)

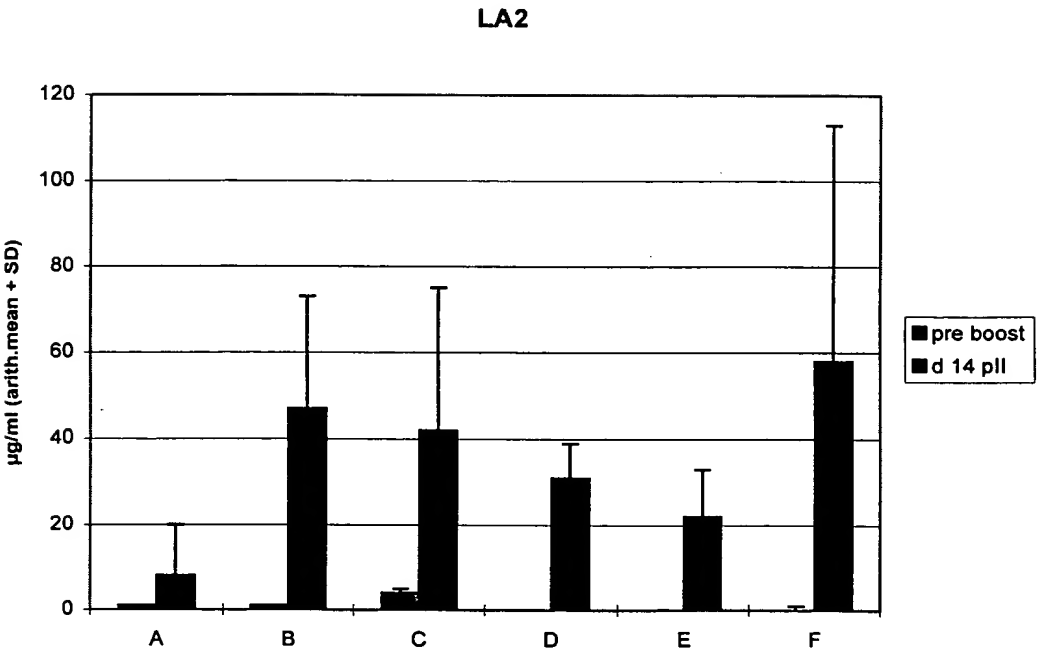

Figure 7, anti-TT immunoglobulin responses as measured by ELISA (see example 5).

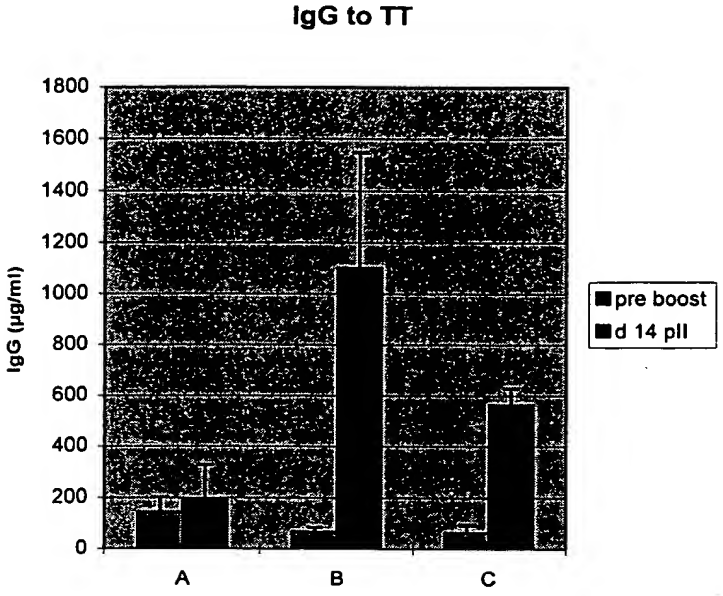

Figure 8, anti FHA immunoglobulin responses as measured by ELISA (see example 5)

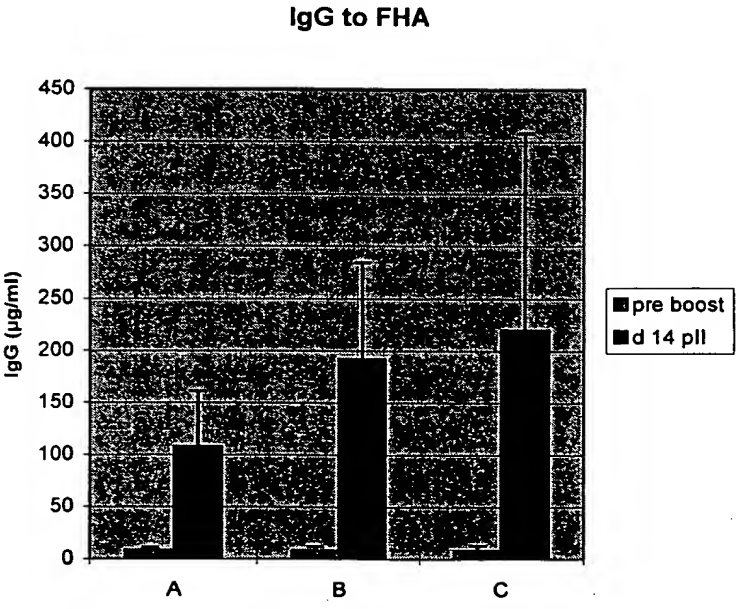

Figure 9, anti-OspA ELISA titres in AGM's (see example 6)

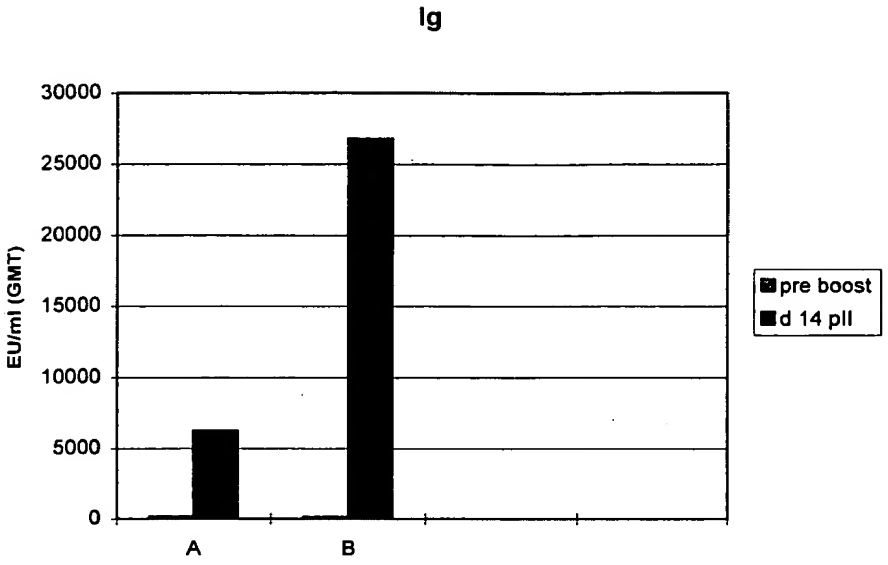

Figure 10, anti-LA2 titres in AGM's (see example 6).

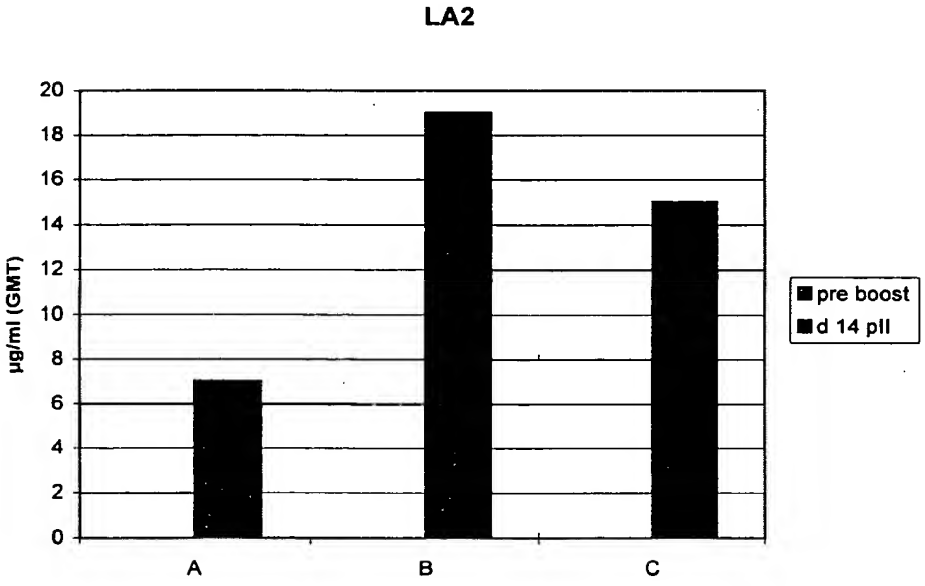

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Figure 11, Intranasal priming and boosting of AGM's, anti-OspA ELISA responses (see example 7).

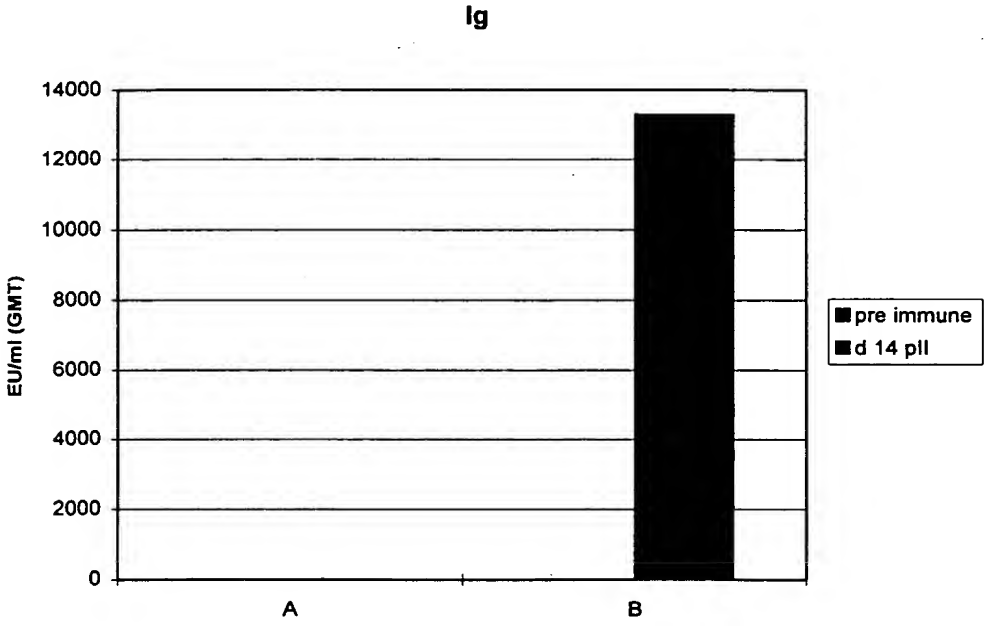

Figure 12, Intranasal priming and boosting of African Green Monkeys with POE and CpG vaccine formulations (see example 8)

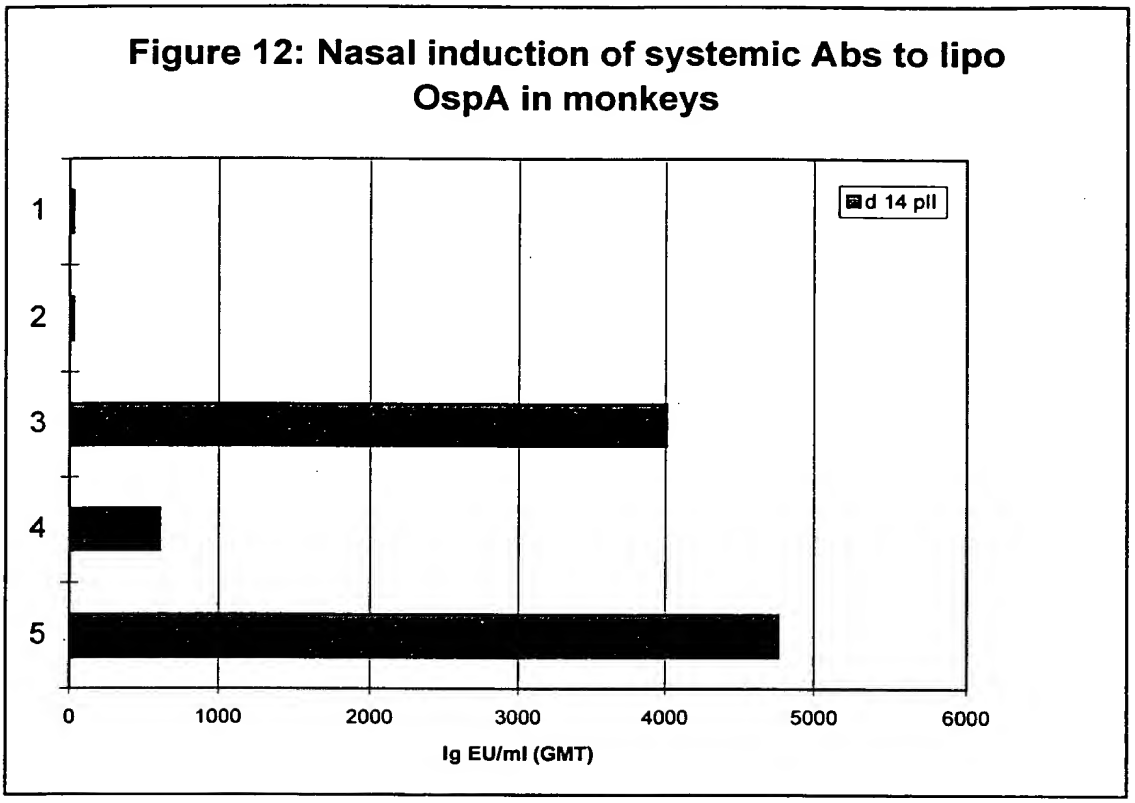

Figure 13, Intranasal priming and boosting of African Green Monkeys with POE and CpG vaccine formulations (see example 8)

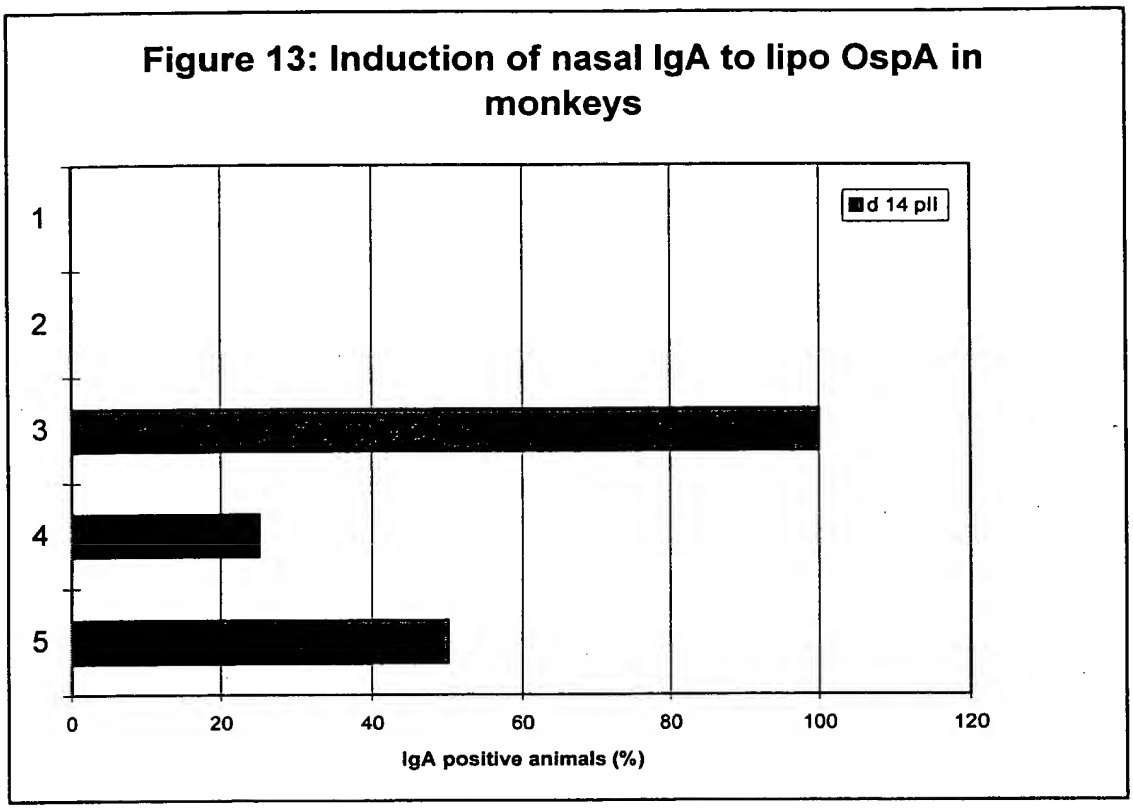

Figure 14, Intranasal priming of mice with Monkeys with PGE and CpG vaccine formulations (see example 9)

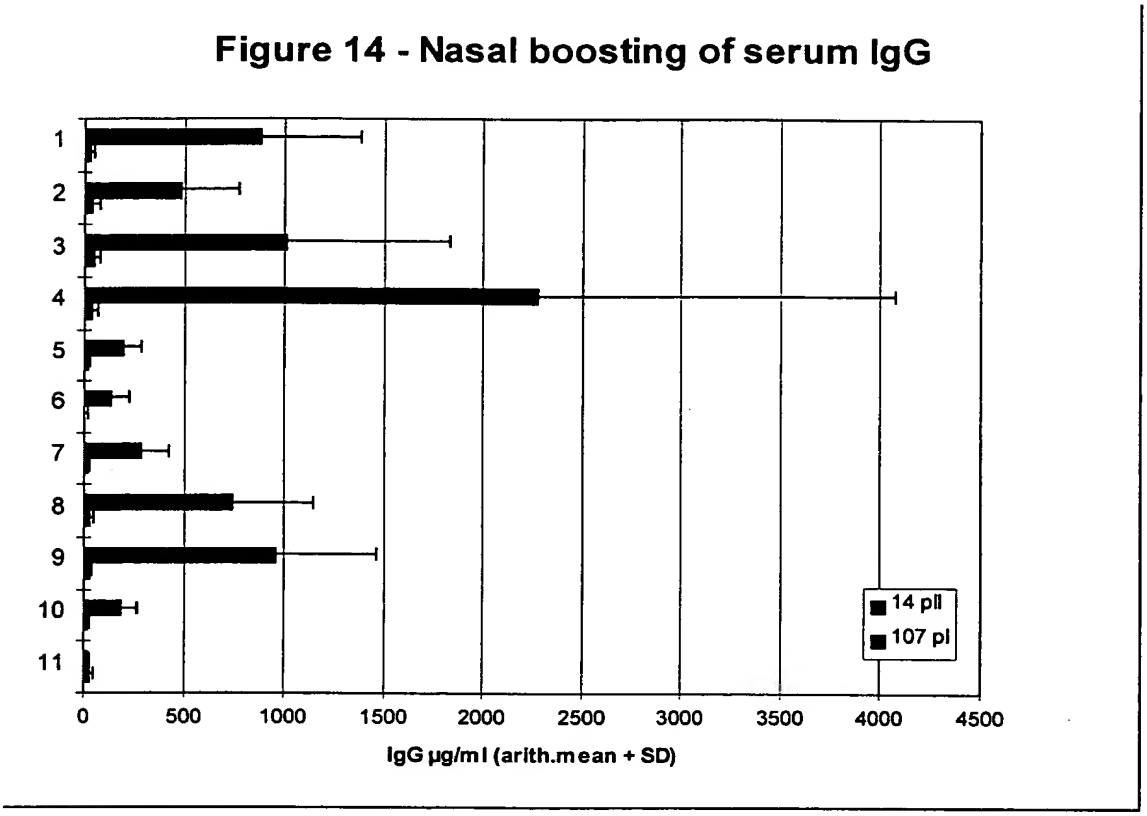

Figure 15, Intranasal priming of mice with Monkeys with POE and CpG vaccine formulations (see example 9)